Micro-Lightguide Spectrophotometry (O2C) for Lower Limb Perfusion: Effects of Exercise Walking in Claudicants

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Abstract

Background Exercise walking has improved walking capacity in patients with intermittent claudication without affecting the macrocirculation reflected in ankle pressures. We wanted to investigate microcirculation in the skin related to exercise walking by using Micro-Lightguide Spectrophotometry (O2C).

Materials and Methods Twenty-eight patients with intermittent claudication—bilateral in 17—were included in a 12 weeks of structured home-based exercise program. The pain-free and maximal walking distances were determined on a treadmill. Saturation and flow, monitored by O2C, were examined immediately before and after the treadmill test. O2C examination took place before as well as after completion of the exercise program. Ankle–brachial index was obtained before treadmill testing.

Results As expected, walking performance improved significantly without affecting ankle pressures. Neither oxygen saturation nor flow, assessed at 2 mm depth, was affected following a 12 weeks of exercise program. We observed a significant decrease in oxygen saturation and flow upon treadmill testing in the both limbs in patients with bilateral peripheral arterial disease (PAD). In contrast, the treadmill test elicited no changes in the opposite and asymptomatic limb in patients with only unilateral PAD.

Conclusion The findings suggest that O2C may be used to study microcirculatory changes. However, it is best suited for the study of phenomena resulting in major changes as it eliminates some inherent variability.

Keywords
- claudication
- atherosclerosis
- ischemia
- lower extremity
- peripheral artery disease

Intermittent claudication (IC) is the classical manifestation of peripheral arterial disease (PAD) caused by atherosclerotic narrowing of the arteries to the lower limb. At rest, the flow demands are met. However, during exercise, blood supply may no longer be sufficient thus eliciting muscle pain in the calf. While resting, the pain subsides but returns with renewed strain. The diagnosis is often based on ankle pressures that are easy to measure with, for instance, a hand-held continuous-wave Doppler device. An ankle–brachial index (ABI) below 0.9 is diagnostic for PAD.[1] In the absence of compressible crural vessels, a toe–brachial index below 0.7 is considered diagnostic.[2]

Management of PAD includes reduction in general risk factors, pharmacological intervention, increased physical activity, and in some instances endovascular or surgical "plumbing."

Several studies have evaluated exercise walking, and the effect on walking distances after a typical 12 weeks of program is well established.[3] Why and how training produces this effect is largely unknown. Exercise has been shown to modify several pathophysiological mechanisms

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in PAD such as cell metabolism, endothelial function, and gait mechanics.

Because of the development in techniques for microvascular investigation, and imaging results from an increasing number of studies, there are indications that PAD also causes dysfunctions in the microcirculation.[4,5] Furthermore, this microvascular dysfunction has been investigated as an early marker of vascular disease even before onset of symptoms.[6]

Micro-Lightguide Spectrophotometry (O2C) is a relatively new, noninvasive, rapid, and pain-free method for assessing microvascular circulation, in patients with PAD. This technique is employed in the "Oxygen 2 See" ("O2C," LEA Medizintechnik, Giessen, Germany) spectrophotometer to measure oxygen saturation (SO_2), relative hemoglobin (rHb), erythrocyte velocity (V), and relative blood flow at 2 and 8 mm depths of skin surface. A study of the technique in a clinical setting has proven it easy to use and with a fair predictive value of a low saturation.[7]

The aim of this study is to investigate, by means of O2C, the microcirculation in patients with IC before and after a treadmill test, and before and after 12 weeks of exercise walking. We hypothesized that a possible ischemia at skin level induced by exercise could be detected with O2C. Furthermore, we hypothesized that an improved walking capacity after a period of exercise walking monitored with O2C would reveal changes in SO_2, flow or both, before or after treadmill testing.

Methods

Patients

The study was performed at the Department of Vascular Surgery at Copenhagen University Hospital Rigshospitalet.

O2C was performed on 30 consecutive subjects who visited the outpatients' clinic due to chronic PAD in Rutherford category 1 through 3. Demographic data was identified by reading the medical journal and asking the subjects. We investigated both legs (60 legs). We identified the most symptomatic limb and marked it as the *index limb*. The least symptomatic limb was marked as *the opposite limb*. One subject could not continue exercise walking due to back-related pain. Another subject was unable to complete the treadmill test on the second visit due to non-PAD-related ankle pain. Thus, 28 subjects were available for analysis.

Informed consent was obtained from every included subject, and the study was approved by the local ethics committee (protocol number: H-3–2012–075).

Methods

O2C: The apparatus used was rented from LEA (LEA Medizintechnik, Giessen, Germany) for the given period. It continuously emits white light (20 W, 500–800 nm, 1-nm resolution) and laser light (830 nm, and $<$30 mW) through a flat probe attached to the skin using double adhesive, transparent tape. The light is scattered in the surface skin layer where it is reflected and collected via the same probe. The light is split into its spectral components and converted to an electrical signal interpreted by a Windows XP operated computer. The white light is used to determine SO_2 and rHb by measuring the amount of light absorbed, and the difference in wavelength on emitted and reflected light due to wavelength-dependent absorption respectively.

The laser light is used to determine erythrocyte velocity from the laser Doppler shift and used to calculate blood flow as a result of rHb and velocity. The premises on which O2C operates have previously been described in detail.

The machine generates velocity, flow, rHb, and SO_2 data 39 times a second resulting in 2340 measure points a minute. For each parameter, we used the median value of all registered data.

Skin Temperature

The tests were performed at room temperature. The temperature of the skin surface was determined before each measurement using an infrared tympanic thermometer (Genius 3000A, Intelligent Medical Systems, Sherwood IMS, Carlsbad, CA). The median temperature was 24.5°C (ICR: 22.5–26.5°C).

Distal Blood Pressure

All blood pressure measurements were performed using standard equipment in the department. They took place in a routine clinical setting for patients with PAD (i.e., an automated sphygmomanometer for brachial pressure and an aneroid sphygmomanometer with an ultrasonic Doppler flow detector for ankle pressure measurements).

Toe blood pressure was measured in diabetic patients only using a photoplethysmograph (Systoe, Atys Medical, France) which detects pulsatile flow and draws a pulse wave curve.

Treadmill

The treadmill test (H/P/Cosmos Mercury, Germany) was initiated at two mph (3.6 km/h) at no inclination increasing to 2% inclination after 5 minutes of walking time, and subsequently increasing 2% every 2 minutes. In a few cases, the subject could not negotiate the speed of 2 mph. Therefore, the test was performed at the individual subject's maximum walking speed, with the same rules for inclination. Registration of the pain free walking distance (PWD) took place when subjects first reported any walking-related pain during the test. Registration of the maximum walking distance (MWD) took place when subjects first reported the inability to walk any further.

Measurement Protocol

All O2C measurements were performed by the same person (TG) as part of an undergraduate scholarship under the supervision of the co-authors. During the examination, the subjects were all in a supine position with their feet at heart level. After resting for a minimum of 5 minutes, each first toe was measured and the participants then performed the treadmill test. After completion, they went back to the examination bed and the probe was repositioned; first on the index limb and then the opposite limb. We planned to let 3 months pass between the first and second visit, but due to logistical reasons, we accepted minor deviations.

Table 1 Demographic data of the 28 patients with intermittent claudication

	Median (P25–P75)	*n* (%)
Age in years	68 (65–74)	28 (100)
Gender	Male/female	19/9 (67/33)
Bilateral symptoms		17 (61)
Diabetes		7 (25)
Coronary artery disease		19 (63)
Pulmonary disease		2 (7)
Smoker status	Current/ previous/never	9/16/3 (32/57/11)

Note: Values are median (interquartile range of 25–75%).

Exercise Walking Protocol

Between the first and second visit, all subjects were instructed to do 30 minutes of walking each day with instructions to extend the distance between their claudication-induced breaks. Exercise was meant to be intense enough to induce claudication pain, and the subject was instructed to continue walking until the pain was moderate. The subject could then rest until the pain subsided and then continue exercising until the moderate claudication recurred.

Exercise walking may be construed differently, ranging from a simple advice of "keep walking" to supervised hospital- or community-based training by medical personnel. Our protocol was positioned between these two extremes and was based on 3 months of structured, home-based exercise walking.

Statistics

Nonparametric statistical analyses were used. Wilcoxon's signed rank test and Spearman's rho correlation coefficient were used for paired data and independent-samples *t*-test was used for unpaired data. The IBM SPSS version 19 (Armonk, NY) was used in performing all statistical analyses in the study. The number of subjects sufficient to cause a significant increase in walking distance was the basis for the sample size. Assuming a 25% improvement from 80 to 100 m walking distance and a standard deviation of 40 m, a type one error risk of 0.05, and a power of 0.8 dictated the need of including 30 patients.

Results

►**Table 1** summarizes the demographic data for the 28 subjects including comorbidities and distal blood pressures.

All patients fulfilled the criteria for PAD corresponding to the index limb and 17 also had PAD corresponding to the opposite limb. We noted a marginally significant improvement in ABI from the first visit (ABI 0.56) to the second visit (ABI 0.65) ($p = 0.048$) (see ►**Table 2**). PWD and MWD increased significantly from the first to the second visit by 39 ($p = 0.02$) and 8% ($p = 0.03$), respectively (►**Table 2**).

SO_2 and flow determined at 2 and 8 mm depth are shown for the index limb as well as for the opposite limb in ►**Table 3**. SO_2 determined at rest at 2 mm was unchanged after 12 weeks of exercise walking (64–66%), as was flow (28–29 AU). Upon treadmill test SO_2 decreased significantly (64–32%; $p < 0.01$) and this response was not modified by 12 weeks of exercise walking (66–38%). Similar results were observed in flow and in SO_2 and flow determined in 8 mm depth (►**Table 3**).

Considering the opposite limb, no significant difference in SO_2 nor in flow was found at either one of the two visits. We then grouped the patients according to the presence of claudication in the opposite limb ($n = 17$) or not ($n = 11$). We noticed a significant decrease in SO_2 in the 17 symptomatic opposite limbs (visit 1 and 2, $p = 0.02$ and 0.001, respectively), whereas no significant changes could be registered in the 11 asymptomatic limbs (visit 1 and 2, $p = 0.3$ and 0.6, respectively) (►**Figs. 1** and **2**).

Focusing on SO_2, determined at 2 mm depth, we registered a significant decrease upon treadmill stressing the index limb ($p < 0.001$), but not in the opposite limb ($p = 0.2$). After the 12 weeks of training, we noticed no changes in SO_2 response to treadmill stressing ($p < 0.0001$) despite the significant change in walking performance.

Table 2 Walk and pressure

	n	Index limb ($n = 28$)	Opposite limb: no symptoms ($n = 11$)	Opposite limb: + symptoms ($n = 17$)
Visit 1				
ABI	25	0.56 (0.50–0.71)	1.00 (0.90–1.02)	0.73 (0.59–0.80)
TBI	3	0.37		
MWD	28	260 (116–422) m		
PWD	28	72 (51–110) m		
Visit 2				
ABI	25	0.65 (0.49–0.77)	1.00 (0.92–1.08)	0.80 (0.56–0.94)
TBI	3	0.37		
MWD	28	287 (175–499) m		
PWD	28	100 (62–165) m		

Note: Ankle–brachial index (ABI), toe–brachial index (TBI), maximal walking distance (MWD), and pain free walking distance (PWD) determined before (visit 1) and after (visit 2) 12 weeks of exercise walking.
Median (interquartile range of 25–75%).

Table 3 O2C results

		First visit		Second visit	
		Before test	After test	Before test	After test
Index limb					
2 mm	SO_2 (%)	64 (54–72)	32 (12–56)	66 (48–71)	38 (8–58)
	Flow (AU)	28 (9–91)	4 (1–16)	29 (8–84)	6 (2–33)
8 mm	SO_2 (%)	57 (47–61)	47 (37–54)	58 (42–66)	41 (34–65)
	Flow (AU)	78 (51–181)	35 (16–68)	135 (44–268)	34 (16–160)
Opposite limb					
2 mm	SO_2 (%)	60 (51–67)	56 (44–67)	66 (59–74)	56 (45–66)
	Flow (AU)	23 (12–68)	29 (11–49)	58 (19–158)	40 (8–76)
8 mm	SO_2 (%	55 (47–59)	55 (45–62)	59 (49–73)	53 (41–65)
	Flow (AU)	75 (55–174)	85 (45–202)	127 (78–300)	108 (43–215)

Note: Oxygen saturation and flow determined at 2 and 8 mm depth before and after treadmill test in the index limb as well as in the opposite limb before (visit 1) and after (visit 2) 12 weeks of exercise walking. SO_2: oxygen saturation; AU: arbitrary units (r_{Hb}/V) . Values are median (interquartile range of 25–75%).

Discussion

The decline of ankle blood pressure after walking is used in the clinical assessment of patients with IC. Standardizing the exertion with a treadmill test enables us to examine the decline and the subsequent recovery of microcirculatory measures.[7] In addition, the same set-up may be used for the study of various modalities in the research of microcirculatory changes following a more or less standardized physiologic stimulus. In a previous study, we found the predictive value of O2C good in cases of low saturation, but in general, insufficient when differentiating normal perfusion from PAD when investigated at rest, which is not surprising as patients suffering from claudication experience no symptoms at rest. Therefore, we set out to investigate O2C by use of two well-known physiologic situations: before and after a treadmill exercise and before and after a 12 weeks of exercise walking.

We had 30 subjects of with PAD suffering from IC of whom 28 completed the planned exercise walking. As expected, their walking distance improved, though less than reported in a recent meta-analysis[8] that suggested an average improvement of 179% for PWD and 122% for MWD following a 6 months of training program.

We did not observe any substantial improvement in distal pressures in spite of significantly improved walking capacity, nor did we find any microcirculatory changes at rest when measured with O2C. Unchanged peripheral pressures upon exercise walking have been reported consistently in the literature, though walking capacity improves. Development of collaterals would be a theoretical explanation for improved gait. In addition, exercise programs and study setups may too be held accountable for the big variability in ABI. Corroborating previous findings, we did not observe any substantial improvement in distal pressures, nor did we find any microcirculatory changes at rest when measured with O2C.

We had hypothesized that the apparatus could detect changes in post-treadmill test saturation and/or flow. These parameters were measured briefly after the maximal walking performance at a time point when the limb perfusion had not yet been restored following the exercise-provoked ischemia (►**Table 3** and ►**Fig. 2**). In practice it is not exactly clear as to how much time had passed exactly before the subjects were investigated, subsequent to the treadmill exercise. Nevertheless, we found a highly significant decrease in the index limb of saturation and flow after both treadmill tests (►**Table 3**). In addition, a similar response was observed in the opposite limb, but only among those who suffered bilateral PAD, in contrast to the group who did not have PAD bilaterally. These results show the anticipated ischemia at skin level post-treadmill testing (►**Figs. 1** and **2**).

We did not see changes in SO_2 nor did we see flow in the values obtained at visit 2 after a 12 weeks of training program. Because we let the patient walk on the treadmill until pain forced them to stop at both visits, maximum ischemia was equally induced at both visits. Hence, our method will not allow us to detect the relevant changes.

With equipment better suited for measuring while the subject is moving, one could obtain insight into the reperfusion curve, which would perhaps differ from the reperfusion curve before the patients went through the exercise walking program. Although easy to use, the O2C apparatus seems to require a rather more static setting.

Regarding the specificity of the O2C apparatus, we found that results from the post-treadmill testing let us separate the group of participants who had bilateral symptoms from those with only symptoms in the index limb, because neither saturation nor flow determined at rest could separate the two groups. The findings indicate that O2C may be used to study microcirculatory changes. But due to the pronounced variability, it is best suited for the study of phenomena resulting in major changes, such as arterial revascularization eliminating some inherent variability.

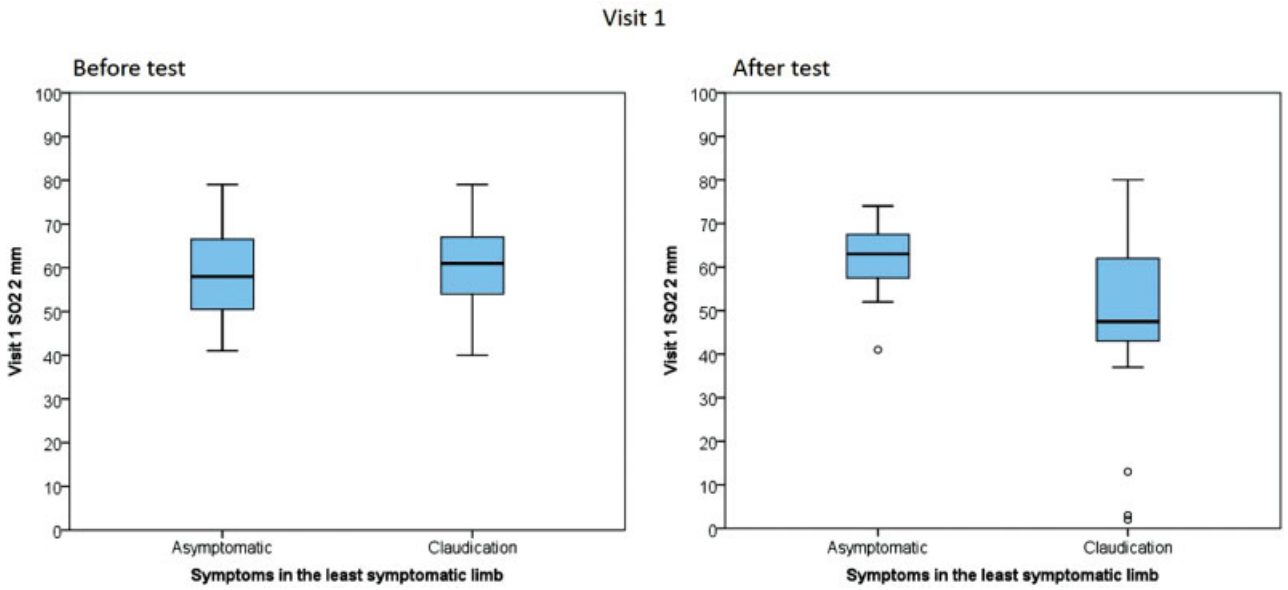

Fig. 1 Boxplot showing median, interquartile range, and range of SO_2 at 2 mm depth before and after treadmill test in the affected (index) and the less affected (opposite) limb, respectively, during the first visit.

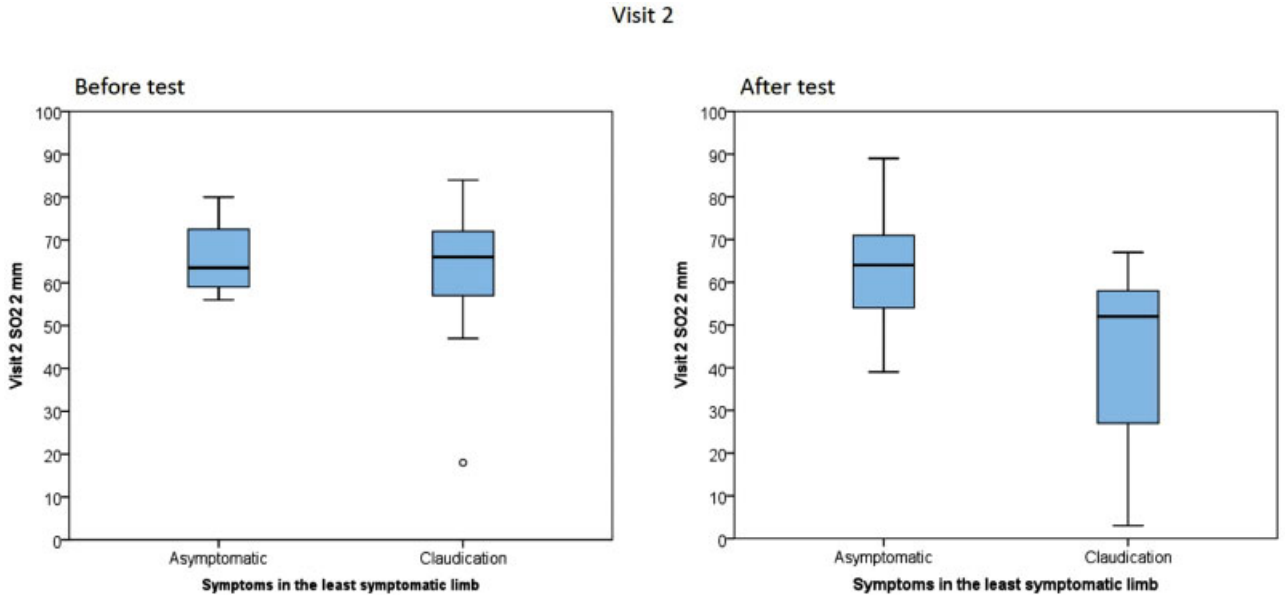

Fig. 2 Boxplot showing median, interquartile range, and range of SO_2 at 2 mm depth before and after treadmill test in the affected (index) and the less affected (opposite) limb, respectively, during the second visit.

Conclusion

The findings indicate that O2C may be used to study microcirculatory changes. We were able to detect changes in the skin perfusion upon ischemia induced by treadmill testing. However, due to the variability it is best suited for the study of phenomena resulting in major changes eliminating some inherent variability.[7] Further studies with measurements being taken on moving object have yet to be explored.

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Conflict of interest
None declared.

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